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The studies we have completed to date suggest that monkey auditory and visual P300s in passive and in active operant conditions exhibit morphological and functional characteristics similar to those observed in human subjects. The role of NA-LC in the genesis of P300 was examined in the present study by recording ERPs in squirrel monkey (Saimiri sciureus) before and after systemic administrations of the alpha-2 adrenergic agonist, clonidine, in doses that are known to suppress the electrophysiological activity of LC neurons. Clonidine significantly decreased the area and increased the latency of P300-like potentials without affecting other ERP components. It also increased EEG power in the alpha range (8-12Hz) and decreased power in the upper beta range (20-40 Hz) which leaving performance unaffected. Administration of clonidine, however, had no effect on the amplitude, area, or latency of the visual P300 component.

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Organization of Technical Report. This report is divided into four sections that correspond to the Specific Aims for Years 04-06. Full-length reports published, accepted for publication, or submitted since the previous Technical Report are listed at the end of this report. The numbers in square brackets in the text refer to the corresponding publication from this list.

AIM 1: TO EXAMINE, IN MONKEYS, THE EFFECTS OF MANIPULATING THE LC-NA SYSTEM ON EVENT-RELATED POTENTIALS (ERPs), ELECTROENCEPHALOGRAPHIC (EEG) CHARACTERISTICS, AND ASSOCIATED BEHAVIORS IN OPERANT PARADIGMS THAT UTILIZE VISUAL OR AUDITORY CUES.

Auditory P300s. The human P300 has been one of the most extensively studied ERP components, and its antecedent conditions are well known. The most common type of P300 occurs following task-relevant stimuli which require a response (Active Paradigms). Other studies have shown that improbable, deviant stimuli that are not task relevant and do not require a response (Passive Paradigms) elicit P300 potentials (P3as) differing in scalp distribution. P3as and P3bs exhibit certain common properties and are thought to be manifestations of information processing functions that are activated by multimodal stimuli, are sensitive to the novelty and/or meaningfulness of the event, reflect perceptual rather than motor functions, and are sensitive to the allocation of task resources. A number of psychological constructs, including context-updating, memory consolidation, orienting, resolution of uncertainty, and stimulus categorization, have been used to explain the variance associated with these events. Thus, P300 is an appropriate ERP measures for studying changes to information processing systems caused by a variety of manipulations including those affecting a particular brain monoaminergic system.

The studies we have completed to date suggest that monkey auditory and visual P300s in passive and in active operant conditions exhibit morphological and functional characteristics similar to those observed in human subjects [2,5]. That is, these are large positive potentials that: a) occur in response to infrequent or novel events embedded in a series of repetitive stimuli; b) begin approximately 150-200 msec after stimulus onset; c) have durations of hundreds of milliseconds; d) peak between 200-400 msec; e) are sensitive to the meaningfulness or relevance of the event; f) reflect the allocation of attentional resources, and g) have corresponding intracranial sources in the hippocampus and cingulate cortex.

Relationships between stimulus intensity and ERP amplitude. We have investigated the amplitude changes that occur in some ERP components with increasing stimulus intensity. In humans, the amplitudes of these components can either increase or decrease in response to increasing stimulus intensity depending on the location of the surface recording site. Large increases characterize components presumably generated by modality-specific sites, while smaller increases, or even decreases, are evident in those originating in associational areas. Comparable data from non-human primates, which would permit invasive studies of the neural substrates underlying these amplitude differences are limited. To more fully characterize intensity-amplitude relationships, auditory ERPs were recorded from chronically implanted epidural electrodes in five squirrel monkeys in response to tones (500 Hz, 300 msec duration) of varying intensities (50, 60, 70, 80 dB SPL). Typically, peak ERP amplitudes recorded at mid-cortical sites (i.e., Fz, Cz, Pz) during the 200 msec interval following stimulus presentation increased substantially with increasing stimulus intensity. In contrast, only small increases or even decreases in amplitude were evident over lateral temporal sites (i.e., T3, T4). These site-specific response profiles exhibited considerable temporal stability. These data suggest that human and monkey exhibit similar responses to changes in stimulus intensity and provide a model to investigate the role of LC-NA in these intensity-amplitude phenomena A report describing these results has been published [1].

Effects of adrenergic agents on monkey P300s: Auditory paradigm. We have continued our pharmacological investigations of a possible role of the noradrenergic (NA) - locus coeruleus (LC) system in the genesis and/or modulation of "cognitive" event-related potentials (ERPs). Pharmacolo 'c activation or suppression of source-cell activity using systemically administered drugs provides another method of studying the relationship between LC activity and the variety of electrophysiological indices that measure cortical information processing. The role of NA-LC in the genesis of P300 was examined in the present study by recording ERPs in squirrel monkey (Saimiri sciureus) before and after systemic administrations of the alpha-2 adrenergic agonist, clonidine, in doses that are known to suppress the electrophysiological activity of LC neurons. In this study, ERPs, EEG, and behavioral performance were recorded in a 90-10 auditory "oddball" paradigm following administration of clonidine or saline placebo. EEG power spectra and behavioral performance were used to assess behavioral state. Clonidine significantly decreased the area and increased the latency of P300-like potentials without affecting other ERP components. It also increased EEG power in the alpha range (8-12 Hz) and decreased power in the upper beta range (20-40 Hz) while leaving performance unaffected. Since clonidine is known to reduce activity in the locus coeruleus and to reduce norepinephrine release, the present results support the hypothesis that NA-LC and its efferent projections are important in modulating auditory P300-like activity in non-human primates. A report describing this experiment has been submitted for publication [4].

Visual P300s. One property of human P300 is that it generally displays similar properties whether it is elicited by auditory, visual, or somatosensory stimuli. One explanation for these similarities is that stimulation from different sensory modalities is channeled to common pathways to engage similar neural mechanisms. The studies just described have shown that an

electrophysiological measure resembling the human P300 potential can be recorded in monkey using an appropriate auditory "oddball" paradigm. In order to investigate the hypothesis that P300-like potentials occur in monkey in response to appropriate visual stimuli, we recorded visual ERPs (VEPs) from chronically implanted electrodes in seven squirrel monkeys (Saimiri sciureus). The results indicate that P300-like potentials can be recorded in a visual "oddball" paradigm. These potentials exhibit sensitivity to stimulus probability and to trial-to-trial changes in stimulus sequence, two properties which characterize the human P300. These observations are significant because they demonstrate that another defining characteristic of the human P300 is also evident in monkey. A report describing these results has been accepted for publication [3].

Effects of adrenergic agents on monkey P300s: visual paradigm. A study was conducted to determine whether the NA-LC system is also involved in the modulation of visual P300-like potentials. In this study, ERPs were recorded from squirrel monkeys (Saimiri sciureus) in a 90-10 visual "oddball" paradigm in which a small, blue rectangle was presented every two seconds on 90% of the trials (background), while a yellow rectangle occurred on 10% of the trials ("oddball"). ERPs were recorded from epidural electrodes before and following systemic administration of clonidine (0.1 mg/kg, IM). Baseline data in response to "oddball" stimuli showed a large, P300-like potential, exhibiting a fronto-central maximum along lateral electrodes. Amplitudes for P3 were larger following 10% than 50% probable "oddball" events. These results suggest that monkeys exhibit large, probability-sensitive, P300-like potentials similar to the visual potentials reported in humans. Administration of clonidine, however, had no effect on the amplitude, area, or latency of the monkey P300 component. This contrasts with our auditory findings. Such differences may reflect different receptor sensitivities or distinct functional roles for norepinephrine in the processing of low-probability acoustic versus low-probability visual signals. It suggests that the role of norepinephrine, as a common neurotransmitter substrate for auditory and visual P300-like potentials, may be oversimplified. A report describing these results has been accepted for publication [3].

Effects of adrenergic agents on monkey P300s: mixed modality (MM) paradigms. To study the role of norepinephrine in multimodal interactions, ERPs were recorded in squirrel monkeys (Saimiri sciureus) using a mixed modality paradigm. In these paradigms both auditory "oddball" (AO) and visual "oddball" (VO) stimuli occurred pseudorandomly in a repetitive sequence of either auditory (MM-auditory paradigm) or visual (MM-visual paradigm) stimuli. Each "oddball" stimulus occurred with 10% probability. In both types of paradigms, P3-like potentials were recorded following AO and VO events. Typically, P3s to VO stimuli were larger in amplitude and longer in latency than those following AO stimuli. Scalp topographies also differed, with P3s to AOs exhibiting distinct centro-parietal distributions while those to VOs exhibited more fronto-central distributions. These differences in scalp topography in the monkey are consistent with recent evidence of modality-specific influences on human P300. The role of the noradrenergic system in the modulation of visual and auditory P3s was examined in another study in which ERPs were recorded in the MM-auditory paradigm before and after the administration of L743,654, an alpha-2 antagonist. These data are in the process of being analyzed.

Studies in progress. Several ERP studies are underway to address the role

of extrathalamic modulation of cortical function in the context of more complex, "cognitive" behavior. We are conducting these studies using cynomolgus monkeys (Macaca fascicularis) since they learn such tasks much more rapidly than squirrel monkeys.

Attention paradigm. Several monkeys are presently being trained to respond to target stimuli in the right or left hemifield and to ignore similar stimuli in the unattended field. Switching of attention is cued by the presence of a large box inside which the stimuli occur. To minimize eye movement artifact, subjects are being trained to fixate a small light in the middle of the screen. We will soon be able to monitor saccadic movements with a video tracking camera.

Face stimuli. Since there are many observations indicating that complex images such as faces are more meaningful to monkeys than simple light flashes or pure tones, we have begun gathering EEG and ERP responses to the presentation of monkey and human faces. We are in the process of training subjects to discriminate between SAME and DIFFERENT categories of faces. Subjects respond to the appearance of either two human or two monkey faces and ignore pairs that are mixed. Human studies have shown that such a task elicits electrophysiological components related to "semantic" processing.

AIM 2: TO CORRELATE THE ACTIVITIES OF INDIVIDUAL MONKEY LC-NA NEURONS WITH CORTICAL NEURONAL ACTIVITY, ERPs, EEG CHARACTERISTICS, AND ASSOCIATED BEHAVIORS IN OPERANT PARADIGMS THAT UTILIZE VISUAL OR AUDITORY CUES.

These experiments are designed to further test the hypothesis that the LC-NA system participates in generating or modulating P300. This involves recording single- and multiple-unit LC activity in untrained and trained cynomolgus monkeys (Macaca fascicularis) while concurrently recording ERPs in an auditory oddball paradigm. The goal was to examine LC unit firing in response to the presentation of standard and target tones and to correlate this activity with the occurrence of P300-like potentials.

Twelve LC recordings were obtained in 3 untrained monkeys during presentation of the auditory oddball paradigm. Ten were isolated single units (SU), while 2 were multi-unit (MU) recordings. One MU recording exhibited a moderate response to infrequent tones but not to frequent tones. Cell firing was significantly elevated from baseline at 44 to 50 msec post-stimulus. ERPs recorded during this session exhibited a small P3a-like potential at the Cz and Pz electrode sites. One single LC cell showed a small but significant phasic response to infrequent tones. Firing rate was increased within the interval of 68 to 86 msec and also from 150 to 154 msec. In the ERPs recorded simultaneously, a P3a was observed at all 3 midline electrode sites. Another cell exhibited a minimally significant SU response to oddball stimuli at 84, 92, and 98 msec. A MU recording from the same region as this latter LC cell, however, failed to demonstrate either a phasic or tonic response to the rare tones. In fact, PSTHs from the remaining 7 single units revealed no phasic or tonic responses to the infrequent stimuli. Several cells outside the LC were recorded during tone presentation. No stimulus-evoked response was elicited from any of these neurons.

Recording sessions are still being conducted in one monkey trained to

perform the auditory discrimination task. Approximately 7 LC recording have been obtained thus far. In 6 of these recordings, the monkey failed to respond, despite performing at a level of 80% correct in baseline ERP sessions. Neither a stimulus-evoked LC response nor a P300 was observed when the monkey failed to attend to the stimuli. One MU LC recording was obtained while the monkey was performing. A preliminary analysis indicated that there may have been a tonic elevation in activity following target tones, as well as a phasic activation related to behavioral response.

AIM 3: TO REPRODUCE AND EXTEND OUR PRELIMINARY OBSERVATION THAT ACTIVATION OF THE LC BY LOCAL DRUG INFUSION, IN HALOTHANE-ANESTHETIZED RATS. PRODUCES EEG SIGNS OF CORTICAL AND HIPPOCAMPAL ACTIVATION.

Effects of LC activation on neuronal activity in somatosensory cortex. A recording/infusion probe was used to activate the neurons of the LC in a reversible and verifiable manner in halothane-anesthetized rats. Infusions of bethanechol increased LC discharge rates 3- to 4-fold for a period of 3-5 min. Simultaneously, recordings were obtained from neurons in the hindlimb region of primary somatosensory cortex. These were activated by appropriate peripheral somatosensory stimuli (air puff or electrical stimulation delivered to the receptive field). Somatosensory responses and background activity were recorded during baseline conditions, LC activation, and LC recovery. Typically, several repetitions of this procedure were conducted for each cortical recording site, and only one recording site was tested per animal. The effects of LC stimulation were highly replicable both within and between animals. Baseline somatosensory responses consisted of a brief, short-latency activation followed by a longer duration pause, in which activity decreased to below background levels, and a gradual return to prestimulus discharge rates. During LC activation, the brief initial response was somewhat reduced, but the previous long-latency reduction in activity became an extended activation. Overall, the absolute magnitude of the total response was increased. Since background activity was reduced during LC activation, the ratio of stimulus-elicited to background activity was considerably enhanced. Thus, the effect of LC stimulation was similar in many regards to that previously reported for iontophoretic application of NE to cortical sensory neurons, although certain differences were also evident. A manuscript describing these results is in preparation.

Effects of LC activation on forebrain electroencephalographic (EEG) activity. In halothane-anesthetized rats, cortical EEG (ECoG) and hippocampal EEG (HEEG) typically exhibit activity similar to that of a lightly sleeping animal. However, periods of "waking" EEG are sometimes observed spontaneously and are always observed following a stimulus such as a tail-pinch, even though the animal is still at a surgical level of anesthesia and does not overtly respond to any such stimulation (all of these phenomena are also observed in humans). We have also utilized the recording/infusion probe to activate the neurons of the LC in halothane-anesthetized rats while simultaneously recording EEG activity in frontal cortex and hippocampus. This experiment has now been completed, with the following findings: 1) LC activation is consistently followed, within 2 to 30 seconds, by ECoG desynchronization and hippocampal theta, 2) if the recording/infusion probe is located so that the infusion is not effective in activating LC neurons (e. g., is placed 1 mm dorsal or ventral to the LC), no such forebrain effects are produced by the infusion, 3) following

infusion-induced activation, forebrain EEG returns to pre-infusion patterns with about the same time course as the recovery of LC activity (10-20 minutes for complete recovery), 4) whether infusions are made from sites medial or lateral to LC, forebrain EEG changes invariably follow LC activation with similar latencies, 5) LC effects on EEG can be blocked by intraventricular administration of the beta-adrenergic antagonist, propranolol. Thus, these data all point to LC activation as a crucial mediating event in producing the EEG effects that follow the bethanechol infusions. A report describing these findings has been published [5].

These observations are especially interesting because they provide evidence that LC activity levels are not only correlated with measures of forebrain activation but can be causally related to cortical and hippocampal EEG patterns. Specifically, LC activation may be sufficient, although possibly not necessary, for forebrain EEG activation.

In order to determine whether LC might be necessary as well as sufficient for forebrain EEG activation, we have now begun also to assess the effects of inactivating LC. The effects of inhibition of LC neuronal activity by peri-LC infusions of the α_2-agonist, clonidine (35-125 nl; 1 ng/nl) were examined in halothane anesthetized rats (n=18). Infusions placed within 400 um of the LC completely inhibited LC neuronal discharge activity within 30-120 seconds, an effect that persisted for 40-100 min. LC neuronal discharge activity gradually returned to preinfusion levels over an additional 30-60 min.

Unilateral infusions had no obvious effects on ECoG activity. Bilateral inhibition of LC activity significantly increased the incidence and amplitude of slow-wave activity in the cortical EEG. Infusions placed 800 um lateral to the LC either completely inhibited LC neuronal discharge activity with a much longer latency of onset (> 4-5 min) and for a much shorter duration, or they produced an incomplete inhibition of LC activity. The onset of the EEG response always followed the complete inhibition of LC activity (bilaterally) within 30-120 seconds, and persisted for the entire period during which LC activity was completely inhibited. Recovery of EEG activity was closely correlated with recovery of LC activity. These results suggest that eliminating LC discharge activity enhances the probability that forebrain sites will exhibit highly synchronized EEG activity.

AIM 4: TO EXAMINE THE RELATIONSHIP BETWEEN THE INTENSITY OF LC NEURONAL ACTIVITY AND RATES OF NOREPINEPHRINE (NE) RELEASE IN NEOCORTEX AND HIPPOCAMPUS BY PERFORMING MICRODIALYSIS IN THESE FOREBRAIN TERMINAL REGIONS IN ANESTHETIZED RATS DURING MANIPULATION OF LC ACTIVITY.

These studies have been initiated (in collaboration with Dr. Ron Kuczenski) and substantial pilot data are available. We have been able to measure NE levels in the cingulate cortex of anesthetized rats using dialysis methods. In general, the quantity of NE in the dialysis samples is near the level of detection. This is sufficient to detect increased NE release, but not decreased release. Locus coeruleus (LC) stimulation (via bethanechol infusion) results in an increase in extracellular NE concentrations in anesthetized rats (n=12). When the magnitude of LC activation is greater than approximately 6 times basal discharge rates, the NE response to a subsequent LC stimulation is absent or attenuated (n=4).

In some cases LC stimulation appeared to lower subsequent basal NE release.

Since these results have been very promising, we have initiated other studies, not proposed in the original application, which now seem worthy of at least some pilot investigation.

Dialysis in unanesthetized rats. We have conducted some feasibility studies of NE dialysis in unanesthetized rats. Results (n=30) have been variable. In some cases NE was present in moderate concentrations and in others NE was present only at low levels. More troublesome however, is that NE levels were not stable during baseline conditions. The source of this variability is not known. One possibility is that there is slight movement of the dialysis probe when the rat is active.

Dialysis in unanesthetized monkeys. A procedure for implanting reference markers in the skull and performing subsequent MRI has been largely worked out. We have ordered a plastic stereotaxic apparatus to be made that can be used in an MRI scanner. The necessary hardware for holding a protective cap to prevent access of the animal to the probes and for holding the head during probe insertion have been designed and machined. We have performed an MRI scan on a euthanized monkey at the Scripps Clinic to pilot out some of the methods we will want to use, and we are now ready to perform an MRI scan on a monkey to be implanted with dialysis probes.

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